Reference: *Biol. Bull.*, **155**: 395–409. (October, 1978)

MICROSPECTROFLUORIMETRIC MEASUREMENTS ON CELLS CONTAINING BIOGENIC AMINES IN THE CEREBRAL GANGLION OF THE POLYCHAETE *NEREIS VIRENS* (SARS)

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The cerebral ganglion of nereid polychaetes performs both neural and endocrine functions. The latter role is poorly documented but behavioral studies (Evans, 1969; 1973) indicate the involvement of the cerebral ganglion in modulation of behavior patterns as well as the importance in behavior of the integration of sensory input from varied prostomial sensory structures (Evans, Cram and Rogers, 1974). The morphology of this ganglion (Holmgren, 1916) indicates massive sensory connections with the anterior and middle levels of the brain, and although neurosecretory elements have not been precisely localized (Marsden, 1978), there is both experimental and histochemical evidence in support of the hindbrain as a primary site of endocrine activity (Clark and Olive, 1973). There is, therefore, a relative wealth (among polychaetes) of information on the nereid cerebral ganglion in terms of microstructure and the kinds of function performed, but very little indeed is known about the biochemical basis of neural activity in this or any other part of the polychaete nervous system.

The nereid cerebral ganglion should, therefore, be a good place to begin studies on the biochemical parameters of the polychaete brain. In this study, on the cerebral ganglion of *Nereis virens*, we have looked for biogenic amines, known to serve as neurotransmitters in many animals, using the techniques of both fluorescence microscopy and microspectrofluorimetric analysis. Previous reports of catecholamines and/or indolylethylamines in the polychaete central nervous system are based on biochemical and fluorescence studies only. They present somewhat varied evidence and, since the optical systems used have not always been clearly defined, the results, in some cases, are hard to evaluate. Microspectrofluorimetric measurements, the surest way of defining the nature of a biogenic amine, have not previously been published for polychaetes.

Materials and Methods

Animals

Nereis virens was obtained from Marine Research Associates, St. Andrews, New Brunswick and was acclimatized to sea water at 11° C. Animals of medium size (3–5 g) were used.

Sections and staining

Paraffin sections, for comparison with the frozen sections used in fluorescence studies, were prepared from prostomia fixed in Bouin's fluid and stained with cresyl

violet, paraldehyde fuchsin or Bodian's protargol silver (Pearse, 1968). As a preliminary step in the localization of the amine-containing cells, 8 living ganglia were stained for 15 minutes at 23° C in a 0.01 mg/ml solution of neutral red in sea water, a vital dye accumulated selectively by aminergic neurons (Stuart, Hudspeth and Hall, 1974).

For fluorescence studies, the prostomium was removed from each of 25 animals anaesthetized in MS222, immediately mounted on a cryostat objective, with O.C.T. compound, placed in commercial propane immersed in liquid nitrogen for 15 to 20 seconds, and then placed in an American Optical Model 840C cryostat set at −35° C. Freezing in liquid nitrogen alone was also tried; no differences in the quantity, discreteness or nature of the fluorescence were seen. Trays of $CaCl_2$ were stored in the cryostat, maintaining a relative humidity between 0 and 30%. Alternate sections (10–12 μ) were used for fluorescence study and as controls. Sections for fluorescence study were kept over P_2O_5 in a closed container in the cryostat for 3 to 48 hours and were then immediately placed on a warming plate to avoid water uptake. They were then kept at 80° C for an hour in a closed jar containing 0.5 g of paraformaldehyde that had been equilibrated in a desiccator over H_2SO_4 at a relative humidity of 50 to 60% (Hamberger, Malmfors and Sachs, 1965). Control sections were treated in the same way except that they were heated in an environment free of formaldehyde vapor. After examination for autofluorescence, control sections were dehydrated and stained with cresyl violet. Freeze-drying of entire prostomia or of supraoesophageal ganglia attached to connective tissue, followed by vacuum embedding in paraffin, was attempted, but abandoned after repeated failure to obtain intact sections. Difficulties with freeze-drying of marine tissues are well known (Welsh, 1972; Stuart *et al.*, 1974).

Microscopy and microspectrofluorimetry

All slides were examined and photographed for specific fluorescence within 24 hours of formaldehyde treatment. Analysis of color and emission spectra of specific fluorescence was carried out on a Leitz Orthoplan microscope equipped with a vertical illuminator consisting of a Ploempak 2 turret system with three combinations of built-in excitation filters, a dichroic-beam splitting mirror and suppression (barrier) filters matched to them (Ploem, 1971). A combination of excitation filter BG3 and S405, dichroic mirror TK 455 and suppression filter K460 gave the best separation of colors, resulting in a cyan (blue-green) for catecholamines and a yellow for indolylethylamines. The filters selected provided excitation energy, with a maximum around 410 nm, reaching the specimen as well as transmission of wave lengths emitted by the specimen above 460 nm. Sometimes a combination of excitation filters BG 12 and KP 5000, dichroic mirror TK 510 and suppression filter K 515 was used for comparison with fluorescence characteristics reported by other authors (Rude, 1966; Myhrberg, 1967). The light source was a high pressure mercury lamp (Phillips CS 100-W2) with a voltage stabilizer.

Emission spectra were measured with an improved Leitz MPV1 unit consisting of the orthoplan microscope as described above as well as an EMI 9558-B photo multiplier tube (S20 cathode) connected to an ultrastabilized high voltage supply, type NSHM BN (Knot Elektronik, München). A Leitz 100× objective and 10×

eyepiece together with a 0.25× projective were used and measurements were made with an aperture of 15 μ^2. A Veril B60 interference graduated filter (Jenaer Glaswerk, Schott and Gen., Mainz, Germany) installed in the measuring beam was used for spectral analysis and a galvanometer recorded intensity. Measurements of photodecomposition (decay of specific fluorescence with exposure to UV light) were taken with the interference filter set at the emission maximum of the fluorescence concerned. The spectral sensitivity of the system was calibrated against a stabilized halogen lamp of known spectral characteristics. Within the spectral range used in this study there was no need to correct emission measurements. As a further check on the reliability of the system, the emission spectrum of a narrow band Hg line 546-10 Schott Model filter was measured once a week. Model droplets of standards were prepared by dissolving 5-HT creatine sulphate and noradrenalin bitartrate in a 2% solution of bovine serum albumin 0.15 M potassium phosphate buffer to a concentration of 0.002 M (Björklund, Nobin and Stenevi, 1971). The solutions were sprayed as droplets on glass slides, dried at room temperature, exposed to formaldehyde and examined by microspectrofluorimetry. Measurements obtained were compared with published spectra for these compounds.

When a reading was taken, the field diaphragm of the illuminating beam was closed until it just surrounded the area of the specimen so that fluorescence emitted by adjacent structures was eliminated. The interference filter was passed across

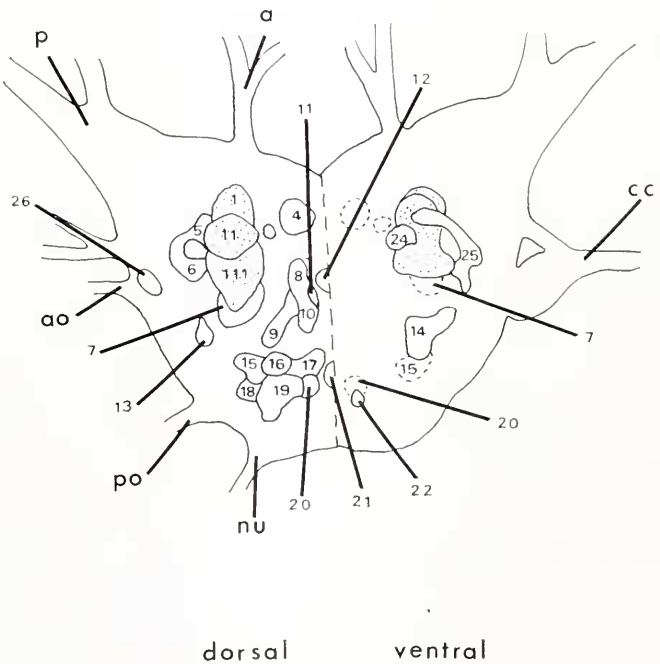

FIGURE 1. Locations of ganglionic nuclei in the cerebral ganglion of *N. virens* (adapted from Holmgren, 1916). a = antennal nerve; ao = anterior optic nerve; cc = circumoesophageal connective, serving cirri; nu = nuchal nerve; p = palpal nerve; po = posterior optic nerve.

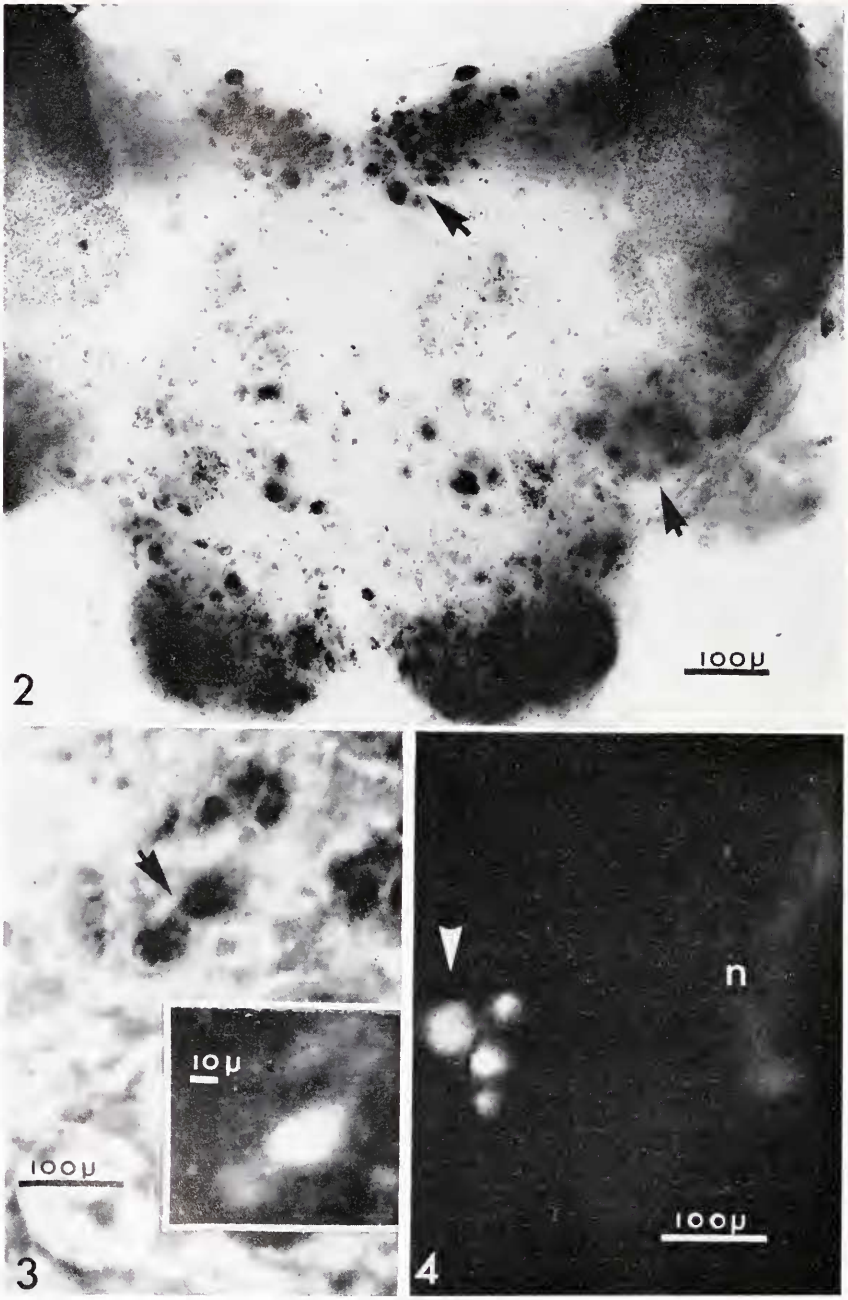

FIGURE 2. Whole mount of cerebral ganglion stained with neutral red. Dorsal view. Arrows indicate nuclei 4 and 14.

FIGURE 3. Nucleus 8: cryostat section, cresyl violet. Arrow indicates cyan fluorescing cells shown in insert.

FIGURE 4. Nucleus 14 showing large (arrow) yellow fluorescent cell adjacent to 3 small cyan fluorescent cells. N shows hazy fluorescence in neuropile.

the emission beam. Galvanometer deflections were recorded at 5 nm intervals. All spectra are expressed as relative quanta versus wave length.

Two tests for specificity of fluorescence were used. The sodium borohydride reduction test (Corrodi, Hillarp and Jonsson, 1964) and the quenching of fluorescence with water.

Cells exhibiting specific fluorescence were assigned to ganglionic nuclei largely by comparing their locations with those of the same cells in adjacent stained sections. Paraffin sections of the same area were also useful. The neuronal nuclei of the cerebral ganglion are numbered according to the system of Holmgren (1916) (Fig. 1). Although the number of fluorescing cells in various parts of each ganglion was recorded, it was decided that a numerical analysis would have little meaning since the exact distribution of fluorescing cells in any one ganglionic nucleus varied from one animal to another, possibly due to cyclic filling and discharge, and the precise localization of any one fluorescing cell in one specific ganglionic nucleus was not always possible.

Results

A cluster of cells in each of two ganglionic nuclei, numbers 4 and 14, stained with neutral red in all cases. In addition a number of single cells stained with neutral red were always scattered over the dorsal side of the ganglion (Fig. 2).

Fluorescence microscopy

A single cerebral ganglion was estimated to contain an average of 45 to 50 cell bodies showing specific fluorescence, 15 to 20 of them yellow fluorescent and 30 to 35 cyan fluorescent. The fluorescence observed was confined to the cytoplasm and could not be followed any distance in the axons. Pin points of cyan fluorescence, probably representing sections of axons, were characteristic of the neuropile, where yellow fluorescence was rare and hazy (Fig. 4). This may be due to the relatively low fluorescent yield of indolylethylamines (Corrodi and Jonsson, 1967). Cyan fluorescent cells (Fig. 3) were present in all ganglionic nuclei exhibiting specific

Table I

Distribution of biogenic amine fluorescence in the prostomium of Nereis virens.

Ganglionic nuclei	Fluorescence		Parts of nervous system	Fluorescence	
	Cyan	Yellow		Cyan	Yellow
4	+	+	Hindbrain	+	+
5	+	−			
6	+	−	Neuropile	Pin points	Haze → neg.
7	+	−	Antennal nerve	+	−
8	+++	+			
9	+++	+	Circumoesophageal connectives	+	−
10	+++	+			
11	+++	+	Cirral ganglion	+	−
14	+	+++	Cirral nerve	+	−
25	+	+	Dorsal epidermis	+	−

fluorescence (Table I), while the yellow fluorescence was more restricted in distribution, occurring most frequently in a few large (25–30 μ), bilaterally symmetrical cells in nucleus 14 (Fig. 4, Table I). Posterior to the neuropile there is a region, containing five nuclei, where one or two fluorescing cells were found in every animal. However, because of the complex structure of this part of the brain, it was impossible to assign these few fluorescent cells to particular nuclei with any degree of confidence.

Specific fluorescence was also characteristic of the prostomial nervous system peripheral to the cerebral ganglion (Table I). Cyan fluorescence was found in groups of cells (15–20 μ) along the circumoesophageal connective, including the

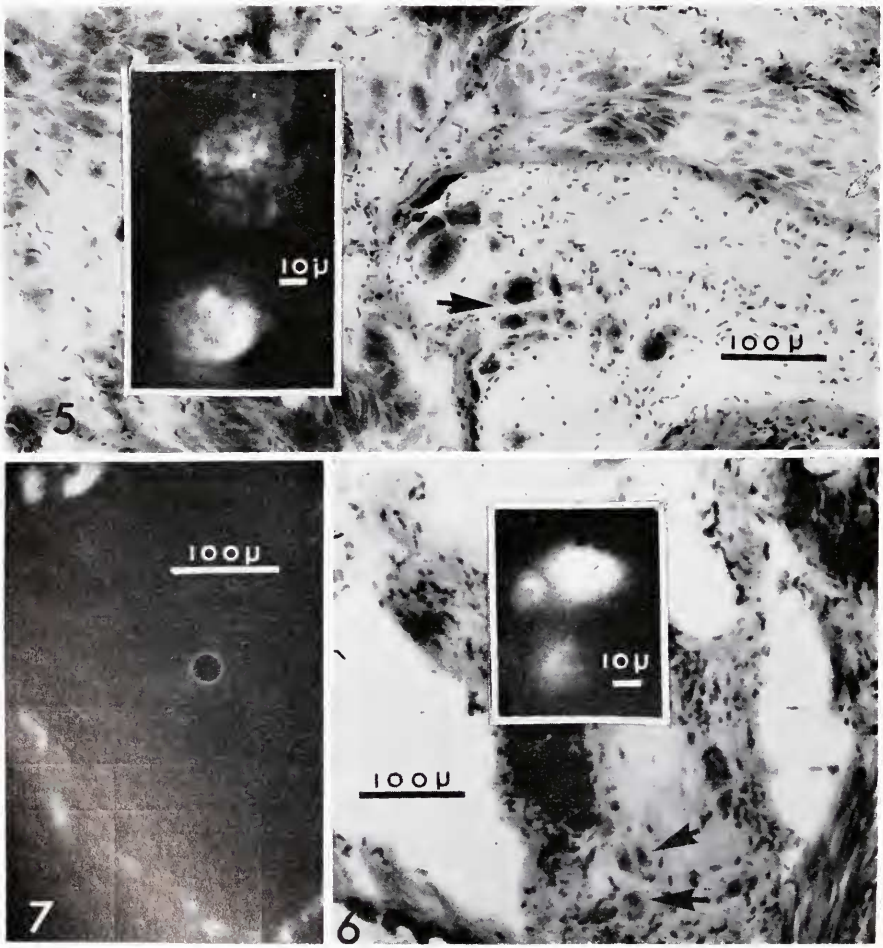

FIGURE 5. Neurons (arrow) in the circumoesophageal connective: cryostat section, cresyl violet. Arrow indicates fluorescing cells shown in insert.

FIGURE 6. Cirral ganglion: cryostat section, cresyl violet. Arrows indicate fluorescing cells shown in insert.

FIGURE 7. Fluorescent bodies around periphery of the base of the antennal nerve.

site of divergence of the cirral nerves, in small cells (10–15 μ) at the base of each cirrus (Fig. 6), faintly along the length of the cirral nerve and in a ring at the base of each antennal nerve (Fig. 7). No fluorescence was seen in this nerve distal to its base and none was found in the optic nerves, nuchal nerves, palpal nerves, tegumentary nerves or in the nerves serving the prostomial musculature. No yellow fluorescence was seen in any part of the prostomium outside the cerebral ganglion. Cyan fluorescent cells were also present in the dorsal prostomial epidermis, in cells which orient perpendicularly to the basement membrane directly above the base of the antennal nerves. They are similar in position and morphology to the fluorescent bipolar sensory cells described by Clark (1966) in *Nepthys* and are probably located among the epidermal sensory cells (Smith, 1957), which in nereids constitute a part of a peripheral sensory system continuous with the antennal nerves. Various forms of autofluorescence were seen in the prostomium of *N. virens* but, with the exception of one case described below, they did not interfere with spectral measurements.

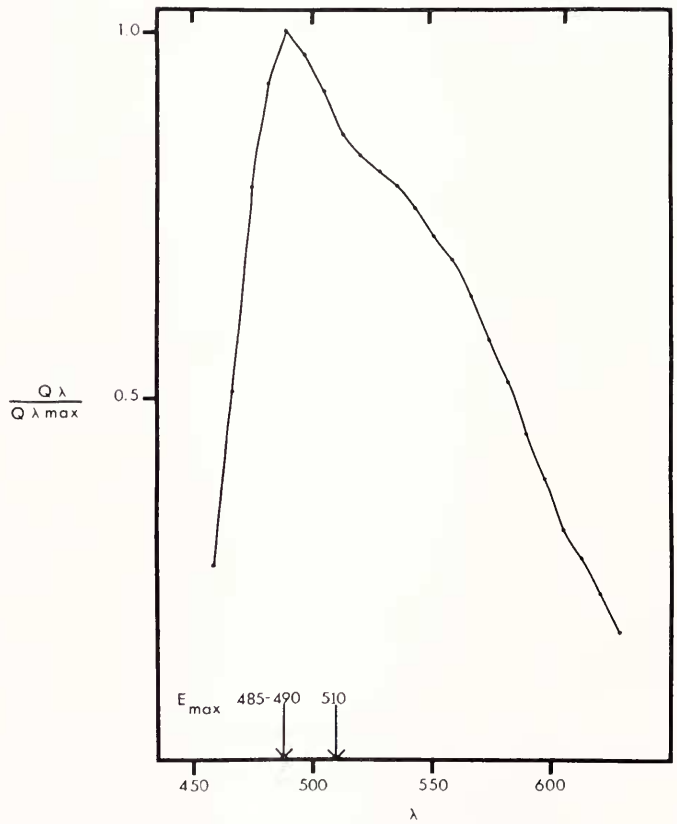

FIGURE 8. Emission spectrum of Type IY yellow specific fluorescence in cerebral neurons.

Microspectrofluorimetry

Yellow fluorescence. Emission spectra obtained from yellow fluorescing cells fall into two categories. Type IY spectra have a well defined maximum at 485 to 490 nm, but also a prominent shoulder in the falling phase, beginning at approximately 510 nm. (Fig. 8) which is presumably responsible for the yellow appearance of this fluorescence. Type 2Y spectra have a very broad emission maximum that extends from 485 to 535 nm (Fig. 9). The emission maxima of these spectra do not correspond with that for any single category of monoamine fluorescence as described by Björklund *et al.* (1971), although the emission maxima of these authors' Group B and Group C fluorophores all fall within the range of both Type IY and Type 2Y spectra. Type IY cells (26 measured) and Type 2Y cells (12 measured) could not be visually distinguished from one another by color and were not confined to separate ganglionic nuclei. In order to determine if the spectra were being distorted by photodecomposition of the fluorophore, readings on 24 cells

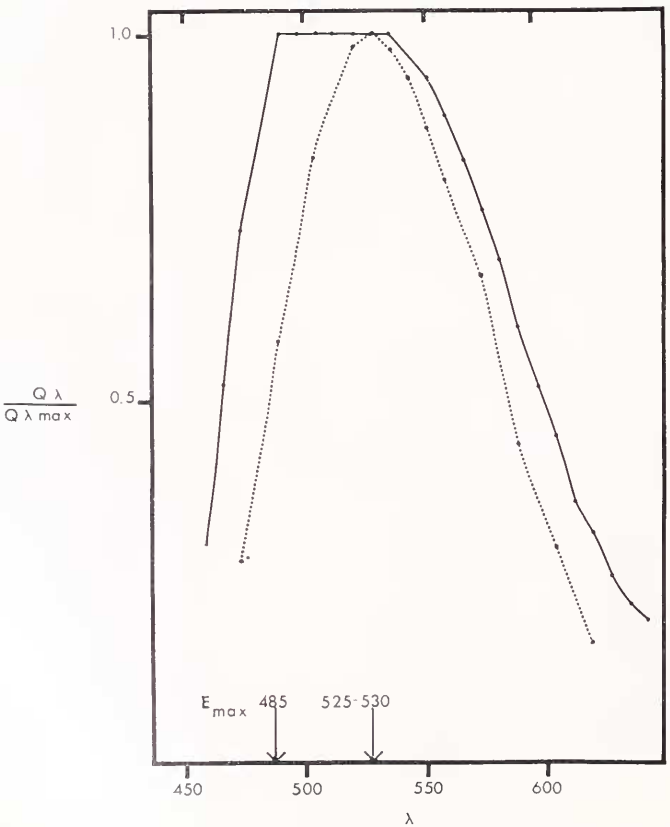

FIGURE 9. Emission spectra of Type 2Y yellow specific fluorescence in cerebral neurons (solid line) and of 5-HT (dotted line) in model droplets (0.002 M).

were taken as soon as the cell had been located and again after five or more minutes of irradiation. No significant alterations of the spectra were observed. The emission maximum for the model droplets of 5-HT was 525 to 530 nm (Fig. 9), which agrees with the maxima reported by others (Ritzén, 1966; Rude, Coggeshall and Van Orden, 1969; Van Orden, 1970; Björklund *et al.*, 1971 and Björklund, Falck and Omen, 1972). Since the maximum of the Type 2Y spectrum extends past the maximum of 5-HT, it is possible that these cells contain 5-HT, although the presence of other formaldehyde-induced compounds excited at 410 nm seems likely. The shoulder of the Type 1Y spectrum approximates the emission maximum for 5-HT, so it is also possible that small amounts of this material occur in the Type 1Y cells. There are a number of indolylethylamines, 5,6-dihydroxytryptamine, 6-hydroxytryptamine, α-methyl-5-hydroxytryptamine and 5-hydroxytryptophan (Jonsson and Sandler, 1969; Björklund *et al.*, 1971) with fluorescence yields comparable to or greater than that for 5-HT and with maxima that fall within the emission maximum of the Type 2Y spectrum. It is possible, therefore, that one or more of these indolylethylamines is contributing to both types of yellow specific fluorescence seen in *N. virens*. In addition, the emission maximum of the Type 1Y cell falls within the maximum (490–500 nm) of the tryptamine fluorophore.

When exposed to UV light indolylethylamine fluorophores decompose more rapidly, except for 6-HT (Jonsson and Sandler, 1969), than do catecholamine fluorophores. Although absolute photodecomposition rates are not available, Casperson, Hillarp and Ritzén (1966) reported that the 5-HT fluorophore fades twice as rapidly as does a catecholamine fluorophore, a relationship which also applies to our yellow *vs.* cyan fluorescence and 5-HT *vs.* noradrenalin standards. These results also imply that 6-HT, which has a high fluorescent yield and fades slowly, is not a major component of either type of yellow fluorescence. However, we found that the photodecomposition rate of the 5-HT standard was distinctly more rapid than that of the yellow cells, due possibly to the fact that the most rapid photodecomposition of the fluorophore of any biogenic amine occurs during the first three minutes of irradiation (Ritzén 1966), so that by the time the cell had been located, the rate of photodecomposition had passed its peak. Alternatively, the fluorophore in the yellow cell body may have an innately slower rate of photodecomposition than does 5-HT. Most fluorophores of indolylethylamines have similar fading characteristics (Jonsson and Sandler, 1969) so that this measure does not serve to differentiate 5-HT from the others. Another possibility, that a shift in catecholamine emission maximum from about 490 to 540 nm, known to occur at high concentrations (Corrodi and Jonsson, 1967), might be contributing to the Type 1Y and 2Y spectra, was investigated by subjecting the sections to a less humid paraformaldehyde and a shorter incubation time, a procedure known to prevent such a change in emission maximum (Jonsson, 1971). With this treatment, the specific fluorescence in the yellow cells was less intense but their spectra had not changed and, in addition, the characteristic emission spectra of adjacent cyan fluorescing cells remained unaltered. The emission spectrum of the autofluorescent glial cells in the cerebral ganglion reaches a maximum at 485 to 490 nm, and extends as a plateau to approximately 565 nm. Spectral readings to determine whether or not glial cells adhering to fluorescent neurons might be contributing to the emission spectra of the specific fluorescence were attempted, but the fluorescent intensity of the glia

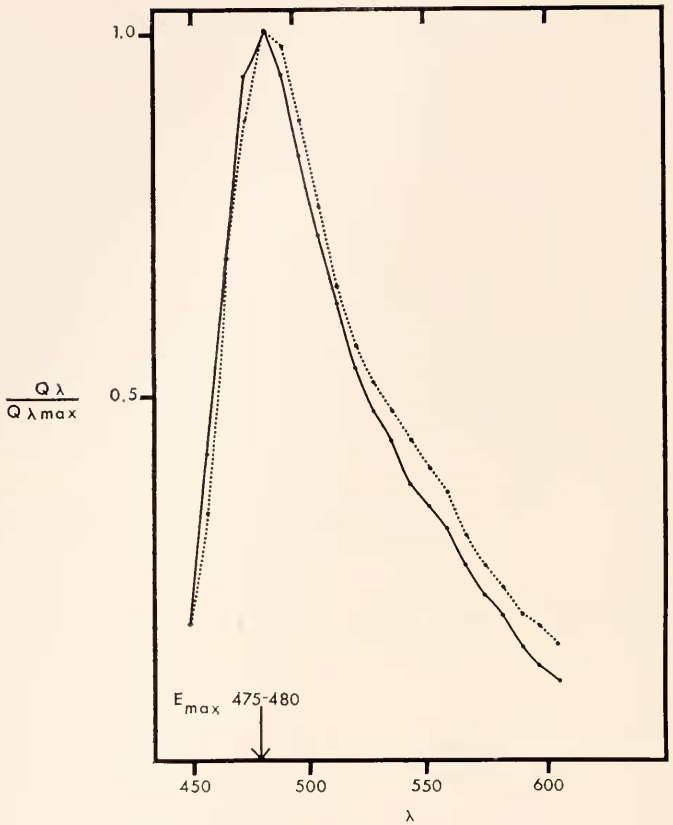

FIGURE 10. Emission spectra of cyan specific fluorescence in cerebral neurons (solid line) and for noradrenalin (dotted line) in model droplets (0.002 M).

was so low that it could not be read by the photomultiplier tube at the amplifications employed for measurement of specific neuronal fluorescence.

Cyan fluorescence. The cyan fluorescing cells and nerve fibers in the cerebral ganglion, circumoesophageal connectives, prostomial nerves and other ganglia of *N. virens* all display an emission spectrum with a well defined maximum at 475 to 480 nm, (Fig. 10), characteristic of the catecholamines dopamine, noradrenalin and adrenalin (Ritzén, 1966; Björklund *et al.*, 1972). However, adrenalin, a secondary catecholamine, requires more energy during dehydrogenation with formaldehyde due to the formation of a quaternary nitrogen in the indole nucleus (Jonsson, 1967), and a longer incubation time may, therefore, be required to bring about the formation of the adrenalin fluorophore (Corrodi and Jonsson, 1967). *N. virens* tissue sections were incubated for an additional 2 hours, but this procedure did not result in new fluorescing structures or in any increase in the intensity of fluorescence. The photodecomposition rate for the cyan fluorescence corresponded more closely to that for the noradrenalin standard than did the yellow

fluorescence to the 5-HT standard, perhaps because catecholamine fluorescence is more resistant to photodecomposition than is indolylethylamine fluorescence. The cyan fluorescence of the epidermal cells was not recorded successfully because it tended to be diffuse and was effectively masked by the autofluorescence (E_{max} 480–490 nm) of the adjacent cuticle.

Tests for specificity. The application of the first step of the sodium borohydride reduction test, the immersion of cryostat sections in 100% ethanol, resulted in the disappearance of all fluorescence. Consequently there was no point in pursuing the second step, the incubation of sections in a solution of $NaBH_4$. The biogenic amines of *N. virens* may be bound only lightly to tissue proteins and may, therefore, be extracted easily by organic solvents. The fluorescence of *N. virens* tissue faded and disappeared when water was added, and after 15 minutes specific fluorescence could not be distinguished from background autofluorescence. Ritzén (1966) reported similar results with noradrenalin and 5-HT fluorophores. When these wet tissue sections were subsequently dehydrated the fluorescence did not reappear.

Discussion

The yellow fluorescent neurons described in this study are similar in distribution to those found in the cerebral ganglion of *Nereis diversicolor* and *N. pelagica* by Warembourg and Dhainaut-Courtois (1969) and Dhainaut-Courtois (1972). This paper, however, provides the first evidence for cyan fluorescent cells in the nereid brain, as well as the first account of the spectral characteristics of yellow and cyan specific fluorescence in a polychaete brain.

The spectral characteristics and photodecomposition rates of the yellow fluorescence, Types 1Y and 2Y, indicate that at least one indolylethylamine, (and probably more), is present. There are, however, at least five known indolylethylamines, including 5-HT, with emission spectra and photodecomposition rates that fall within the parameters of our fluorophores (Jonsson and Sandler, 1969). One particularly likely candidate is tryptamine (E_{max} 490–500 nm), which fits fairly well with the peak of the Type 1Y spectrum. Tryptamine is a precursor of all indolylethylamine derivatives and it is possible that the 1Y and 2Y spectra reflect two distinct metabolic conditions in the yellow neurons, with tryptamine in particularly high concentrations in Type I. Since all 'yellow' neurons fall clearly into either Type I or Type II categories, they presumably represent either two phases of one sort of neuron or two distinct kinds of neuron. Bufotenine, suggested by Dhainaut-Courtois, Caridroit and Bizerte (1969) as the source of a specific yellow fluorescence with an emission maximum (no spectral curve published) at 525 nm in the nervous systems of *N. diversicolor* and *N. pelagica,* cannot be seriously considered, since this substance is a tertiary amine and should not, therefore, fluoresce (Corrodi and Jonsson, 1967; Björklund *et al.*, 1971). Yellow fluorescing cells with a broad emission maximum have also been reported for *Hirudo* (Ehinger, Falck and Myhrberg, 1968), *Lumbricus* (Ehinger and Myhrberg, 1971) and the regenerating nervous system of *Allolobophora* (Koritsánszky and Hartwig, 1974). These few microspectrofluorimetric studies present a situation similar to that found in *N. virens* and suggest that the spectral characteristics of the yellow fluorescence in annelid nervous sysems are likely to be complex and may involve a number of indolylethylamines.

The Retzius cells of the leech, on the other hand, appear to contain 5-HT only (Rude *et al.*, 1969), and biochemical analysis of the ventral nerve cord of *Arenicola marina, Amphitrite ornata, Glycera dibranchiata* (Welsh and Moorehead, 1960) and *Glycera convoluta* (Manaranche and l'Hermite, 1973) have shown the presence of significant amounts of 5-HT. It seems likely, therefore, that this amine may be one component of the yellow specific fluorescence seen in *Nereis*.

We have several clues as to the function of indolylethylamines in annelids. Recently Marsden and Lacalli (1978) have shown that 5-HT appears to stimulate mucus release by larvae of *Arenicola cristata*. Lent (1973) argued that the Retzius cells control mucus secretion by the skin of the leech, and has suggested (1977) that mediation of secretion may be an evolutionarily ancient rôle for 5-HT. Coleman (1975) disputes Lent's position and considers it more probable that the 5-HT in the Retzius cell serves to modulate the action of other, probably cholinergic, neurons exciting the mucous cell A related argument has been proposed by Alvarez, del Castillo and Sanchez (1969), who found that 5-HT appeared to modify the excitatory effect of ACh on longitudinal body wall muscle in the sabellid *Sabellastarte*. There may, perhaps, be some phylogenetic significance to the fact that large, yellow fluorescing neurons are characteristic of the ventro-lateral nucleus 14 in *Nereis diversicolor, N. pelagica* (Warembourg and Dhainaut-Courtois, 1969), *N. virens*, and the ventro-lateral region of the cerebral ganglion of the related genus *Nephtys* (Clark, 1966). In *N. virens* there are also smaller numbers of such cells on the dorso-medial nuclei 4, 10, and 11. All these cell groups lie close to the roots of bundles of nerve axons passing to prostomial palps, tentacles and cirri richly supplied with mucous cells. The absence of peripheral yellow fluorescence, which appears to argue against indolylethylaminergic innervation of mucous glands, may, as mentioned earlier, be an artifact caused by rapid fading. Peripheral destinations for processes from central 'yellow' cells, although undemonstrated in *Nereis*, may exist. Yellow specific fluorescence has been seen in the antennal nerves of both *Nephtys caeca* (Clark, 1966) and *Glycera convoluta* (Manaranche and l'Hermite, 1973). Also, since the cerebral ganglion of *Nereis* is almost certainly involved in habituation and learning (Evans, 1969; Evans *et al.*, 1974) and neurosecretion, (Clark and Olive, 1973) an interneuronal role of some kind for indolylethylaminergic neurons within the cerebral ganglion is a reasonable possibility, although one we are far from being able to substantiate.

The spectral characteristics of the cyan fluorescence found in the cerebral ganglion and prostomial nerves of *Nereis virens* indicate that a catecholamine is present. Although biochemical and fluorescence histochemical studies on other nereids (*N. pelagica* and *N. diversicolor*) have failed to demonstrate the presence of catecholamines (Warembourg and Dhainaut-Courtois, 1969; Dhainaut-Courtois, 1969; 1972), our discovery of catecholamines in *N. virens* is in accord with biochemical studies on *Glycera* (Manaranche and l'Hermite, 1973), bioassays on *Arenicola* (Östlund, 1954), as well as with microspectrofluorimetric measurements for *Lumbricus* (Ehinger and Myhrberg, 1971), *Allolobophora* (Koritsánszky and Hartwig, 1974) and *Hirudo* (Ehinger *et al.*, 1968). Since all catecholamines and their α-methylated derivatives and corresponding amino acids (DOPA and -m-DOPA) have the same spectral characteristics (Jonsson, 1967), it is not possible, from our evidence, to say which substance(s) is present. However, our

analysis indicates that adrenalin is unlikely. The biochemical studies on *Arenicola marina* (Östlund, 1954) and *Glycera convoluta* (Manaranche and l'Hermite, 1973) suggest that noradrenalin and dopamine are possibilities.

Although there is no experimental evidence for a functional rôle for catecholamines in annelids (Gerschenfeld, 1973), the cyan fluorescence in prostomial epidermal cells along the cirral and at the base of the antennal nerves implies catecholaminergic pathways which correspond morphologically with an epidermal sensory system characteristic of nereids (Smith, 1957). Catecholaminergic cells, interpreted as sensory, have also been observed in the prostomial integument in *Lumbricus* (Dahl, Falck, von Mecklenburg and Myhrberg, 1963) and *Nephtys* (Clark, 1966). Within the cerebral ganglion, although there are probably some sensory cells, such as those in nucleus 18 (Whittle and Zahid, 1974), it seems unlikely that the rather substantial numbers of catecholaminergic neurons, most of them not in the vicinity of nucleus 18, are all sensory. It is more probable, particularly in view of the abundance of cyan fluorescence in the neuropile, that most of the cerebral catecholaminergic cells serve some collecting, controlling or modifying purpose. In this connection the presence of aminergic neurons in the hindbrain, in close proximity to probable neuro-secretory cells, is interesting and deserves further study. Therefore, although some of our findings are compatible with the fairly widely considered hypothesis (Dahl *et al;* 1963; Clark, 1966; Myhrberg, 1967; Marsden and Kerkut, 1969 and Welsh, 1973) that in annelids catecholaminergic neurons are sensory and indolylethylamine-containing cells are motor, it seems likely that at least some of the aminergic neurons located in the cerebral ganglion of *N. virens* belong to the third category of interneurons and function in a variety of different ways.

This research was supported by an Operating Grant from the National Research Council of Canada to Joan Marsden and made possible by the technical expertise of Robert Lamarche, whose help is gratefully acknowledged.

Summary

1. Vital staining with neutral red reveals that possible biogenic amine-containing cells are abundant in the cerebral ganglion of the polychaete, *Nereis virens*.

2. Fluorescence microscopy indicates two kinds of cells exhibiting specific fluorescence, one blue-green (cyan) and the other yellow.

3. The emission spectrum recorded from the cyan fluorescing cells is characteristic of a catecholamine, which is probably not adrenalin.

4. Emission spectra recorded from yellow fluorescing cells are of two types, both suggestive of a mixture of indolylethylamines. It is probable that 5-HT is one component of these spectra. A possible relationship between some of these yellow cells and prostomial mucous glands is discussed.

5. Yellow specific fluorescence was confined to the cerebral ganglion where cyan fluorescence was also seen in peripheral areas, a distribution suggestive of a sensory rôle for peripheral catecholaminergic cells.

6. The probability that both catecholaminergic and indolylethylamine-containing neurons in the cerebral ganglion serve to control or modify other neural or neurosecretory activity is discussed.

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